THE CAUSE OF GREEN COLORATION OF BACTERIAL COLONIES IN BLOOD-AGAR PLATES.*

GUSTAV F. RUEDIGER.

(From the Memorial Institute for Infectious Diseases, Chicago.)

IN 1903 Schottmüller,[1] and very soon afterward Rosenow,[2] showed that pneumococci produce green colonies in blood-agar plates, while *Streptococcus pyogenes* produces small, grayish, hemolyzing colonies. Schottmüller showed that there are streptococci which produce green colonies in these plates very similar to the pneumococcus colonies. I have found pneumococci which produce hemolyzing colonies similar to those of *Strept. pyogenes.*

In making use of blood-agar plates for the purpose of studying the streptococci and diplococci found in throats, I have noticed a number of very interesting peculiarities of some cultures. A small number of strains of streptococci were found which produce the characteristic hemolyzing colonies in plates made of plain agar and 0.4 c.c. of blood, and green colonies of the pneumococcus type in plates made of glucose agar and blood. Furthermore, it was found that a large number of typical *Strept. pyogenes* cultures produce no hemolysis in plates made of glucose blood-agar, although they produce very extensive hemolysis in plates made of plain agar and blood. If these glucose blood-agar plates are allowed to remain in the incubator for 36 to 48 hours, the streptococcus colonies become distinctly green and are surrounded by a green halo. In some instances the green coloration is observed at the end of 24 hours.

These facts led me to inquire into the cause of the green coloration, and the idea presented itself that it is in some way connected with the production of acid by the colonies. This idea was then tested, and all the facts which have been ascertained point to the correctness of the view. Sugar-free agar was prepared by dissolving 0.5 per cent NaCl, 1 per cent peptone (Witte), and 1.5 per cent agar-agar in distilled water, clarifying, tubing, and sterilizing in the autoclav.

* Received for publication June 1, 1906.

[1] *Münch. med. Wchnschr.*, 1903, 50, pp. 849, 909.

[2] *Jour. Infect. Dis.*, 1904, 1, p. 308.

It was found now that pneumococci which produce distinctly green colonies in ordinary plain blood-agar, or glucose blood-agar plates do not produce more than a faint trace of green in plates composed of sugar-free agar and 0.3 to 0.4 c.c. of fresh defibrinated rabbit blood. But if 1 per cent of glucose, lactose, or inulin is added to the sugar-free agar, these cultures again produce deep green colonies in blood-agar plates made with it. It is necessary that the cultures should rapidly ferment inulin if they are to produce green colonies in inulin blood-agar plates.

On the other hand, it has been found that *Strept. pyogenes*, which does not readily ferment the polysaccharides, produces hemolyzing colonies in inulin, dextrin, and usually in lactose blood-agar plates, although it forms green colonies in plates of glucose blood-agar.

Several other cultures were tested, and it was found that *Staph. aureus* and *B. typhosus* produce distinctly green colonies in glucose blood-agar plates, although there is no trace of green in the plates made of plain agar and blood. *B. coli communis* was also tested, and it was found that this organism produces rapid and extensive hemolysis in all blood plates, and hence no green coloration could be detected.

It might be asked now why *Strept. pyogenes* does not produce green colonies in ordinary plain blood-agar plates. This is probably due to two reasons: (1) these organisms probably do not readily ferment the muscle sugar which is found in plain agar, and (2) they produce such rapid hemolysis that the green would not be detected, because we can see no green coloration after the red corpuscles have been completely hemolyzed.

It is quite certain from these experiments that the green coloration of bacterial colonies in blood-agar plates is dependent upon the production of acid. As all the cultures which have been worked with produce lactic acid in glucose broth (as shown by the ferric chloride test), it is very probable that the green color is caused by the action of lactic acid on the red corpuscles or on the hemoglobin. It cannot yet be stated whether the green color is due to a blending of colors or to the production of a pigment, possibly bilivirdin. Further work must be done on this point.

It must be mentioned that pneumococcus colonies produce dif-

ferent degrees of green coloration in plates made of sugar-free agar and different kinds of blood. Whereas there may be no trace of green with one sample of blood, in plates containing blood from a different animal the colonies may have a decided greenish tint. This may possibly be due to the slight variation in the sugar content of the blood.

CONCLUSIONS.

The green coloration of bacterial colonies in blood-agar plates is dependent upon the production of acid and the action of this acid (probably lactic acid) on the red corpuscles.

B. coli communis produces extensive hemolysis in blood-agar plates.